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In vitro Inhibition of Human Hepatic and cDNA-expressed Sulfotransferase Activity with 3-Hydroxybenzo[*a*]pyrene by Polychlorobiphenylols

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Running Title: Hydroxy-PCBs inhibit sulfonation of 3-OH-BaP

Key words: polychlorobiphenylols; 3-hydroxy-benzo(a)pyrene; inhibition of sulfonation; SULT1A1*1 and SULT1A1*2; human liver cytosol; SULT1E1

Abbreviations: BaP, benzo[*a*]pyrene; BaP-3-SO_4, benzo[*a*]pyrene-3-sulfate; CB, chlorobiphenyl; CYP, cytochrome P450; OH-PCB, polychlorobiphenylol; 3-OH-BaP, 3-hydroxybenzo[*a*]pyrene; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SULT, sulfotransferase; T4, thyroxine; TTR, transthyretin. Abbreviations for individual OH-PCBs are as recommended in Maervoet et al. 2004.

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Abstract

Sulfonation is a major phase II biotransformation reaction. We showed that several polychlorobiphenylols (OH-PCBs) inhibited the sulfonation of 3-hydroxybenzo(a)pyrene (3-OH-BaP) by human liver cytosol, and some cDNA-expressed sulfotransferases. At concentrations above 0.15 μM, 3-OH-BaP inhibited its own sulfonation in cytosol fractions that were genotyped for SULT1A1 variants, as well as with expressed SULT1A1*1, SULT1A1*2 and SULT1E1, but not with SULT1A3 or SULT1B1. The inhibition fit a two-substrate kinetic model. We examined the effects of OH-PCBs on the sulfonation of 0.1 or 1.0 μM 3-OH-BaP, respectively non-inhibitory and inhibitory substrate concentrations. At the lower 3-OH-BaP concentration, OH-PCBs with a 3-chloro-4-hydroxy-substitution pattern were more potent inhibitors of cytosolic sulfotransferase activity, with IC_{50} values between 0.33 and 1.1 μM, than OH-PCBs with a 3,5-dichloro-4-hydroxy-substitution pattern, which had IC_{50} values from 1.3 to 6.7 μM. We found similar results with expressed SULT1A1*1 and SULT1A1*2. The OH-PCBs were considerably less potent inhibitors when assay tubes contained 1.0 μM 3-OH-BaP. The inhibition mechanism was non-competitive, and our results suggested the OH-PCBs competed with 3-OH-BaP at an inhibitory site on the enzyme. The OH-PCBs tested inhibited sulfonation of 3-OH-BaP by SULT1E1, but the order of inhibitory potency was different than for SULT1A1. SULT1E1 inhibitory potency correlated with the dihedral angle of the OH-PCBs. The OH-PCBs tested were generally poor inhibitors of SULT1A3 and SULT1B1-dependent activity with 3-OH-BaP. These findings demonstrate an interaction between potentially toxic hydroxylated metabolites of PCBs and polycyclic aromatic hydrocarbons, which could result in reduced clearance by sulfonation.

Introduction

Polycyclic aromatic hydrocarbons (PAHs)[1] and polychlorinated biphenyls (PCBs) are two classes of environmentally prevalent pollutants. PAHs are formed through the combustion of fossil fuels and the burning of organic materials (Dipple 1985). PCBs were produced industrially in the middle of the last century for their desirable dielectric properties (Erickson 2001) and remain in the environment due to their continued use, their release from waste sites and because many congeners are slowly degraded. The more lipophilic PAHs and PCBs are often found in the same environmental samples, such as soils and sediments, and are biotransformed in animals by similar pathways (James 2001).

Of the PAHs, benzo[*a*]pyrene (BaP) is a well-studied chemical carcinogen, which is metabolized by cytochrome P-450 (CYP) to a variety of products (Dipple 1985). These include 3-hydroxybenzo[*a*]pyrene (3-OH-BaP), a major metabolite of BaP in humans and animals, which has estrogenic properties and binds to hemoglobin (Charles et al. 2000, Sugihara and James 2003). Hydroxylated PAH metabolites such as 3-OH-BaP are substrates for glucuronidation and sulfonation, catalyzed by one or more of the UDP-glucuronosyltransferases (UGTs) and PAPS-dependent sulfotransferases (SULTs), respectively (James et al. 2001)**.** Sulfonation is considered a detoxication pathway for 3-OH-BaP.

PCBs have several metabolites of toxicological importance, including the polychlorobiphenylols (OH-PCBs), which are formed *in vivo* from CYP-dependent monooxygenation of PCBs (James 2001). Although they are slightly more hydrophilic than the parent PCBs, several OH-PCBs are eliminated slowly (Klasson-Wehler et al. 1993). People who are highly exposed to PCBs through the diet typically have OH-PCBs in their blood, some bound to plasma proteins (Sandau et al. 2000, Guvenius et al. 2003). Several OH-PCB congeners

interact with components of the endocrine system, potentially interfering with thyroid hormone and estrogen function (Safe 1994; Lans et al. 1993; Schuur et al. 1998). Although the OH-PCBs have low affinities for both α and β estrogen receptors, some OH-PCBs were strikingly potent inhibitors of human estrogen sulfotransferase (SULT1E1), with sub-nM IC_{50} values (Kester et al. 2000). This suggested that OH-PCBs may be indirectly estrogenic, by increasing estradiol bioavailability in target tissues. As well as possibly causing toxicity by inhibiting the sulfonation of hormones, several OH-PCBs inhibited the sulfonation and glucuronidation of the PAH metabolite 3-OH-BaP in channel catfish intestine (van den Hurk et al. 2002).

Sulfonation is an important phase II conjugation pathway for the detoxification of xenobiotics as well as the modulation of endogenous compounds such as thyroid hormones, steroids, and neurotransmitters (Coughtrie et al. 1998). One or more members of a superfamily of cytosolic SULT enzymes catalyze these reactions (Blanchard et al. 2004). SULT1A1, SULT1B1 and SULT1E1 are the major phenol sulfotransferases expressed in human liver, with SULT1A1 (also known as ST1A3) found at the highest concentration (Honma et al., 2002). SULT1A3 is expressed in the gut, but is present in very low concentrations in adult human liver (Richard et al. 2001). Genetic polymorphisms are known for SULT1A1: a $G^{638} \rightarrow A$ transition leading to an $Arg^{213} \rightarrow$ His exchange in the protein was observed with a frequency of 33.2% in Caucasian subjects, 8% in Chinese, and 29.4% in African-American (Carlini et al. 2001). SULT1A1*His (SULT1A1*2) was a less thermostable protein than SULT1A1*Arg (SULT1A1*1) and some authors have reported the *2 variant was less catalytically active (Raftogianis et al. 1997; Ozawa et al. 1998).

Because people are frequently co-exposed to PAH and PCB, we wished to determine if OH-PCBs would inhibit 3-OH-BaP sulfonation in human liver cytosol, and if so, whether or not

the inhibition was isozyme selective. We used cDNA-expressed human SULT1A1*1, 1A1*2, 1A3, 1B1 and 1E1 isozymes, which we expected would utilize 3-OH-BaP as substrate. We genotyped the human liver cytosol fractions used in this study with respect to the common SULT1A1 polymorphic variants, to examine the possibility that OH-PCBs would affect their activity differently. These studies were conducted with a series of predominantly *para*-OH-PCBs.

Materials and Methods

Materials. The structures of the OH-PCBs used in this study are shown in Figure 1. In naming these OH-PCBs we followed the recommendation of Maervoet et al. (2004), to name them as metabolites of PCBs, referring back to the Ballschmiter and Zell numbering system for PCBs. The 6′-OH-CB35 (**A1**), 4′-OH-CB35 (**B1)**, 4′-OH-CB36 (**B2**), 4′-OH-CB79 (**C1**), and 4-OH-CB36 (**C2**) were synthesized by the Suzuki-coupling as described previously (Bauer et al. 1995; Lehmler et al. 2001). We verified the structures of each of these OH-PCBs by ^{1}H-, ^{13}C-NMR, FT-IR and GC-MS spectroscopy. We found that each OH-PCB was >99% pure by GC-MS analysis (Mass Spectrometry Facility of the University of Kentucky, Lexington, KY), combustion analysis (Atlantic Microlab, Atlanta, GA), and thin layer chromatography. The 4′-OH-CB69 (**B3**), 4′-OH-CB106 (**B4**), 4′-OH-CB112 (**B5**), 4′-OH-CB121 (**C3**), 4′-OH-CB159 (**C4**), 4′-OH-CB165 (**C5**) and 4′-OH-CB72 (**C6**) were purchased from AccuStandard, Inc., New Haven, CT. Dr. S.S. Singer, University of Dayton, OH supplied the 3′-phosphoadenosine-5′-phosphosulfate (PAPS). We purchased ^{35}S-PAPS, 3.05 μCi/nmol (99.1% pure) from Perkin Elmer Life Science, Inc., Boston, MA. Benzo[*a*]pyrene-3-sulfate (BaP-3-SO_4) and 3-hydroxybenzo[*a*]pyrene (3-OH-BaP) were purchased from the NCI Chemical Carcinogen Reference Standard Repository (Midwest Research Institute, Kansas City, MO). We obtained *Hae*II from Fisher Scientific (Atlanta, GA), and *Taq* DNA polymerase, along with other polymerase chain reaction (PCR) reagents from Promega (Madison, WI). Integrated DNA Technologies, Inc. (Coralville, IA) supplied primers for use in genotyping. We purchased the highest available grade of other reagents from Fisher Scientific, Atlanta, GA, and Sigma, St. Louis, MO.

Physical-chemical properties of the OH-PCBs. We calculated the structural characteristics of dihedral angle, molecular volume, molecular surface area, p*K*a, log P and log D at pH 7.0 with MM2* using GB/SA water solvent continuum as implemented by MacroModel 5.0, and described previously (Tampal et al. 2002).

Cytosolic Preparations. Dr. F. P. Guengerich, Vanderbilt University kindly donated the samples of human liver, which were procured from organ donors (Guengerich, 1995). We prepared liver cytosolic fractions from four livers by standard methods and stored aliquots at −80°C until use (Wang et al. 2004). We used three to four of these cytosol fractions in each experiment.

SULT1A1 genotype determination. We used a genomic DNA isolation kit (InVitrogen) to extract genomic DNA from samples of the individual human livers used in this study. We used a published method to detect the SULT1A1 polymorphism status of each liver (Ozawa et al. 1998; Nowell et al. 2000). Amplified DNA fragments were digested with *Hae*II and the fragments resolved on 2% (weight/volume) agarose gels. Fragments from individuals homozygous for SULT1A1*1 exhibited two bands, visualized by ultraviolet trans-illumination, while DNA fragments from individuals homozygous for SULT1A1*2 were not cleaved by this enzyme and exhibited one band.

Expression and partial purification of SULT isozymes. Previous work described the expression in *E. coli* of human SULT1A1*1, SULT1A3, SULT1B1 and SULT1E1 (Dajani et al. 1998; Wang et al. 1998). We grew *E. coli* cells containing the respective sulfotransferase genes as described previously, and prepared 100,000 g supernatant fractions for use in inhibition studies and for partial purification of the SULT enzymes. We purchased expressed SULT1A1*2 cytosolic extract from PanVera, Madison, WI, and used it as provided.

The 100,000 g supernatant fractions of SULT1A1*1, SULT1A3, SULT1B1 and SULT1E1 were partially purified by previously published chromatographic methods (Falany et al. 1990; 1994). Following the last step (PAP-agarose affinity column) we dialyzed the fractions eluted with PAP with three changes of buffer to remove PAP before assay of SULT activity with 3-OH-BaP as substrate. We analyzed active fractions by SDS-PAGE (Laemmli 1970) to assess the purity of each SULT enzyme. We stained the gels with Coomassie R-250 reagent and determined the percentage of protein present as each respective SULT enzyme by scanning densitometry.

Kinetic analysis of 3-OH-BaP sulfonation. We determined SULT activity with 3-OH-BaP as substrate by a fluorimetric assay of BaP-3-SO_4 product formation, as described previously (Wang et al. 2004). We ensured that the formation of BaP-3-SO_4 was linear for time and protein, and did not exceed 10% of the added 3-OH-BaP with each of the enzyme sources used. Duplicate tubes were prepared for each incubation condition. We examined the kinetics of sulfonation in three liver cytosol fractions by systematically varying the concentration of 3-OH-BaP or PAPS. When the variable substrate was 3-OH-BaP, we used 12 concentrations in the range 0.035 to 2.00 μM, and the concentration of PAPS was kept constant at 10 μM. When we varied PAPS, we used 7 concentrations between 0.157 to 10.0 μM, and kept the concentration of 3-OH-BaP constant at 0.100 μM.

We determined the kinetic parameters for 3-OH-BaP sulfonation by partially purified preparations of the cDNA-expressed SULT isozymes under incubation conditions similar to those used for liver cytosol. For SULT1A1*1 and 1A1*2, we used 7 substrate concentrations in the range 5 to 100 nM; for SULT1E1 we used six 3-OH-BaP concentrations between 15.6 to

1000 nM; and for SULT1A3 and 1B1 we used seven concentrations of 3-OH-BaP, 0.25 to 5.0 μM.

Inhibition of SULT activity by OH-PCBs. To assess inhibition of 3-OH-BaP SULT activity, we prepared stock solutions of the OH-PCBs in DMSO, and added aliquots to incubation mixtures such that the final concentration of OH-PCB was in the range of 0.01 to 200 μM and the DMSO concentration did not exceed 0.5% (v/v). For each OH-PCB, we examined the concentration dependence of inhibition with three liver cytosol fractions, as well as with cytosol fractions from the *E. Coli* expressing SULT1A1*1, SULT1A3, SULT1B1 and SULT1E1, and the purchased Sf-9 cytosol fraction containing SULT1A1*2. For studies with human liver cytosol, SULT1A1*1 and SULT1E1, we examined two concentrations of 3-OH-BaP, 0.1 μM and 1.0 μM. For studies with SULT1A1*2, we examined only 0.1 μM 3-OH-BaP, a concentration that did not elicit substrate inhibition. For studies with SULT1B1, we used only 1.0 μM 3-OH-BaP, since this enzyme had very low activity at 0.1 μM 3-OH-BaP, and did not exhibit substrate inhibition. Examination of the effect of 50 μM concentrations of several OH-PCBs on the activity of SULT1A3, measured with 1.0 μM 3-OH-BaP, revealed little inhibition, so no further concentrations were studied.

Kinetics of inhibition. To study the type of inhibition produced by OH-PCBs, we used 4′-OH-CB112 (**B5**) as a model inhibitor. We prepared four sets of assay tubes containing human liver cytosol and varying amounts of 3-OH-BaP from 35 to 150 nM: one set (control) contained no 4′-OH-CB112; the other sets contained 0.25 μM, 0.5 μM or 1.0 μM 4′-OH-CB112.

Data analysis. We calculated the enzyme kinetic parameters from studies with variable concentrations of 3-OH-BaP using non-linear regression analysis and GraphPad 4.0 software (San Diego, CA). We selected the built-in Michaelis-Menten equation for most analyses. Where

we found evidence of 3-OH-BaP substrate inhibition, we fit the data into an equation derived from a two-substrate model (Zhang et al. 1998), $V=V_{max1}(1+(V_{max2}S/V_{max1}K_i))/(1+K_m/S+S/K_i)$. This equation denoted the constant for binding of the first substrate molecule as K_m and the second substrate molecule as K_i.

We calculated the effects of OH-PCBs on 3-OH-BaP SULT activity as percentage inhibition compared to the controls without inhibitor. We obtained IC_{50} values by fitting log OH-PCB concentration and % control activity to a sigmoidal curve. We examined the relationships between IC_{50} and physico-chemical properties of the OH-PCBs by linear correlation analysis. We calculated the inhibitory constant (K_i) from the kinetic studies with 4′-OH-CB112 by means of Dixon plots and plots of K_m/V_{max} against inhibitor concentration (Cornish-Bowden, 1995).

Results

SULT1A1 genotype of the liver donors. We found that the human liver cytosols used were from individuals with different SULT1A1 genotypes, as determined by PCR amplification of the region of the SULT1A gene flanking the polymorphic base pair. The G to A mutation in SULT1A1 removed the restriction site for the endonuclease *Hae*II. As shown in Figure 2, an individual homozygous for the SULT1A1*2 allele did not have the *Hae*II restriction site and the PCR product was not cleaved (Lane 1). The PCR product from the individual homozygous for SULT1A1*1 showed complete cleavage by *Hae*II, generating two fragments of approximately 100 and 181 bp (Lane 3). Enzymatic digestion of the PCR product from the heterozygote (SULT1A1*1/*2) generated one band of 281 bp and the two fragments of 100 and 181 bp (Lane 2). Thus, the individual designated HL 1 was homozygous for the *1 allele, HL 2 was heterozygous and HL 3 was homozygous for the *2 allele.

Sulfonation of 3-OH-BaP by human liver cytosol and expressed human SULT isoforms. Initial studies of the sulfonation of 3-OH-BaP by human liver cytosol revealed that concentrations of 3-OH-BaP above 0.15 μM resulted in a decrease in activity. To find a saturating concentration of PAPS, we conducted incubations in the presence of 0.1 μM 3-OH-BaP and varying concentrations of PAPS. The data fit the Michaelis-Menten equation, with apparent K_m of 0.56 ± 0.09 μM and V_{max} of 48 ± 2 pmol/min/mg protein (mean ± S.D., n= 3). The dependence of activity upon PAPS concentration in expressed human SULT1A1*2, in the presence of 0.1 μM 3-OH-BaP, also followed Michaelis-Menten kinetics. The apparent K_m was 0.32 μM and V_{max} was 684 pmol/min/mg protein. As shown in Figure 3, cytosol and the expressed enzyme were saturated by a PAPS concentration of 10 μM, and we used this concentration in subsequent studies.

We conducted detailed studies of the effect of a range of 3-OH-BaP concentrations up to 2 μM on reaction rates with human liver cytosol and expressed human SULT1A1*2. We obtained preliminary estimates of the kinetic constants K_m and V_{max1} by fitting the initial rates of sulfonation at concentrations below 0.15 μM 3-OH-BaP to the Michaelis-Menten equation. We then obtained the values of K_i and V_{max2} through constraining K_m using the equation of Zhang et al. (1998). We also analyzed data by constraining V_{max1}, but a better fit was found when constraining K_m. Figure 4A shows how the data fit this equation for three individual human liver cytosols. Kinetic studies with expressed SULT1A1*2 revealed substrate inhibition with the single enzyme (Figure 4B). Table 1 shows values for K_m, K_i, V_{max1} and V_{max2} for each human liver cytosol and the expressed SULT1A1*2. The expressed enzyme showed a lower value for K_m (0.022 μM) and K_i (0.16 μM) than any of the human liver cytosols.

Table 2 shows the results of kinetic studies with the other expressed human enzymes. The values shown in Table 2 are from substrate concentration ranges in which the data fit the Michaelis-Menten equation. SULT1A1*1 and SULT1E1 showed substrate inhibition at concentrations of 3-OH-BaP above 0.15 μM, but detailed kinetic analyses at inhibitory concentrations was not conducted with these expressed enzymes. It was found that SULT1A1*1 had a similar apparent K_m (0.018 μM) to that found with SULT1A1*2 (0.022 μM). SULT1E1 also had high affinity for 3-OH-BaP, with an apparent K_m of 0.05 μM. SULT1A3 and SULT1B1 did not exhibit substrate inhibition over a concentration range up to 5 μM, and showed much higher apparent K_m values for 3-OH-BaP. These expressed enzyme preparations were partially purified, and SDS-PAGE showed they contained different percentages of the respective SULT enzymes, as stated in the footnote of Table 2. The values shown for V_{max} were corrected for the percentage of each respective SULT isoform in the partially purified enzyme preparation.

Inhibition of 3-OH-BaP sulfonation by OH-PCBs with Human Liver Cytosol The 4-OH-PCBs with one (**B** group) or two (**C** group) flanking chlorine substituents inhibited human liver cytosolic 3-OH-BaP sulfotransferase activity in a concentration-dependent manner. Figure 5A shows inhibition curves from selected OH-PCBs in the presence of 0.1 μM 3-OH-BaP, and Figure 5B shows the same compounds studied with 1.0 μM 3-OH-BaP. Table 3 presents the concentrations that produced 50% inhibition (IC_{50}) of 3-OH-BaP sulfotransferase activity with all the tested compounds, at each of two concentrations of 3-OH-BaP. Compounds **B1**-**B5** with the 3-chloro-4-hydroxy-substitution pattern were potent inhibitors, with IC_{50} values ranging from 0.33 to 1.08 μM, when activity was measured with 0.1 μM 3-OH-BaP. The OH-PCBs with two chlorine atoms flanking the hydroxy group (**C1**-**C6**) were less potent as inhibitors under these conditions (IC_{50}: 1.31-6.71 μM) (Table 3). The single 6-OH-PCB studied, **A1**, was a very weak

inhibitor, with an IC_{50} of > 100 μM (Figure 5). When activity was measured with 1 μM 3-OH-BaP, a concentration at which substrate inhibition occurred, the measured IC_{50} values showed lower inhibitory potencies for all OH-PCBs, but especially so for the **C** group compounds, whose IC_{50} values ranged from 3 to 58.7 μM (Table 3).

Inhibition of 3-OH-BaP sulfonation by OH-PCBs with cDNA-expressed SULTs.

*SULT1A1*1* Figure 6A shows inhibition curves with selected OH-PCBs using 0.1 μM 3-OH-BaP, whereas Figure 6B shows results with a substrate concentration of 1.0 μM 3-OH-BaP. We found that 6′-OH-CB35 (**A1**) was a poor inhibitor of 3-OH-BaP sulfonation under both conditions of substrate concentration. When using 0.1 μM 3-OH-BaP, type **B** compounds (**B1**-**B5**) showed IC_{50} values ranging from 0.77 to 1.31 μM, whereas type **C** compounds (**C1**-**C6**) exhibited IC_{50} from 2.16 to 6.65 μM (Table 3). When using 1.0 μM 3-OH-BaP, the inhibitory potencies of the OH-PCBs were dramatically reduced. The IC_{50} concentrations for **B**-type OH-PCBs were reduced to 10.3 - 67.5 μM, and for **C**-type OH-PCBs were 33.8 to more than 100 μM (Table 3). *SULT1A1*2* The IC_{50} of 6′-OH-CB35 (**A1**) was more than 100 μM, as shown in Table 3. At 0.1 μM 3-OH-BaP concentration, the IC_{50} of **B**-type compounds (**B1**-**B5**) ranged from 0.54 to 1.48 μM, and 1.67-6.52 μM for **C**-type compounds (**C1**-**C6**). When using 1.0 μM 3-OH-BaP concentration, the concentration of OH-PCB causing 50% inhibition was around 5 μM for type **B** (**B1**-**B5**) compounds and 50 μM for type **C** (**C1**-**C6**) compounds (data not shown). *SULT1A3* As shown in Figure 7, this expressed enzyme was not inhibited or was weakly inhibited by OH-PCBs when using 3-OH-BaP as substrate at the non-inhibitory concentration of 1.0 μM. Addition of 50 μM concentrations of compounds 6′-OH-CB35 **(A1**), 4′-OH-CB69 (**B3**), 4′-OH-CB106 (**B4**), 4′-OH-CB112 (**B5**), 4′-OH-CB121 (**C3**), 4′-OH-CB165 (**C5**) and 4′-OH-CB72

(**C6**) did not inhibit the sulfonation of 3-OH-BaP. Compound **B1** (4′-OH-CB35), 4′-OH-CB36 (**B2**), 4′-OH-CB79 (**C1**), 4′-OH-CB159 (**C4**) showed 2-20 % inhibition at 50 μM, and 4-OH-CB36 (**C2**) produced 43% inhibition. Since SULT1A3 activity was poorly inhibited by 50 μM concentrations, we did not examine a range of concentrations of OH-PCBs. *SULT1B1* This expressed enzyme showed a quite different inhibitory interaction with OH-PCBs, compared with SULT1A1*1, 1A1*2, 1A3 and 1E1, in that 6′-OH-CB35 (**A1**) was a quite potent inhibitor (IC_{50}: 4.72 μM) of 3-OH-BaP sulfonation (Table 3). Compounds **B1** (4′-OH-CB35) and **B4** (4′-OH-CB106) showed IC_{50} values of 16.76 and 17.45 μM, respectively. The other type **B** and **C** OH-PCBs were weak inhibitors. *SULT1E1* Compound **A1** (6′-OH-CB35) was a poor inhibitor of 3-OH-BaP sulfonation at either of the substrate concentrations studied (Table 3). When using 0.1 μM 3-OH-BaP concentration, OH-PCBs with no or one *ortho* substituted chlorine (**B1**, **B2**, **B4**, **C1**, **C2**, **C4** and **C6**) were potent inhibitors of 3-OH-BaP sulfonation, with IC_{50} values between 0.24 and 1.3 μM (Table 3). The OH-PCBs with two *ortho* substituted chlorine atoms (**B3**, **B5**, **C3** and **C5**) were less potent inhibitors, with IC_{50}: 4.87-7.98 μM (Table 3). When we used 1.0 μM 3-OH-BaP as substrate, there was a three to five-fold reduction in inhibitory potency, and the order of potency remained as it was with 0.1 μM 3-OH-BaP.

Structure-activity relationships. For human liver cytosol, expressed SULT1A1*1, SULT1A1*2 and SULT1E1 we investigated the relationship between inhibitory potency, measured at 0.1 μM 3-OH-BaP, and each of several physical-chemical properties of the 4-OH-PCBs. For human liver cytosol, SULT1A1*1 and SULT1A1*2, we found no significant correlation between dihedral angle, molecular surface area, molecular surface volume, log P, log D at pH 7.0 or p*K*a. The IC_{50} values with SULT1E1 showed a significant ($p<0.001$) linear

correlation with dihedral angle as shown in Figure 8. No other significant correlations were found.

Kinetics of 3-OH-BaP sulfotransferase inhibition by 4'-OH-CB112. We investigated the type of inhibition of 3-OH-BaP sulfonation using human liver cytosol. Figure 9A shows that 4′-OH-CB112 (**B5**) reduced sulfotransferase activities at all the tested 3-OH-BaP concentrations in a concentration-dependent manner. The kinetic constants showed a steady reduction in V_{max} with increasing concentration of 4′-OH-CB112, but little change in K_m, indicating a non-competitive type of inhibition (Table 4). Figure 9B shows a plot of K_m/V_{max} versus the concentration of 4′-OH-CB112 which indicated a K_i value for 4′-OH-CB112 of 0.52 ± 0.14 μM.

Discussion

The major human metabolite of benzo(a)pyrene, 3-OH-BaP, was very readily sulfonated in human liver cytosol, especially at concentrations below 0.15 μM. We observed substrate inhibition in human liver cytosol and with SULT1A1 and SULT1E1, but not SULT1A3 or SULT1B1. We studied the kinetics of substrate inhibition in liver cytosol and SULT1A1*2, and found they fit a two-substrate model proposed for the sulfonation of estradiol by SULT1E1. This model suggested that SULT1E1 could bind two molecules of estradiol per molecule of enzyme, one in a preferred site for sulfonation and the other at an allosteric site associated with substrate inhibition (Zhang et al. 1998). Our results suggest a similar scenario for the interaction of 3-OH-BaP with SULT in human liver cytosol and SULT1A1. The K_m values for each of the three tested human liver cytosol fractions (48 to 51 nM), SULT1A1*2 (22 nM) and SULT1A1*1 (18 nM) were low, indicating that 3-OH-BaP has a very high affinity for human SULT1A1. The K_i values were about 10-fold higher. The 3-OH-BaP was not, however, specific for SULT1A1, but

was a substrate for the other human phenol sulfotransferases studied. In particular SULT1E1 showed a high affinity for 3-OH-BaP, with K_m of 50 nM. A related compound, 1-hydroxypyrene, also had a very low K_m with SULT1A1, 8 nM, and SULT1E1, 21 nM, but a higher K_m with SULT1A3 of 0.8 μM (Ma et al. 2003). When we calculated 3-OH-BaP clearance values (V_{max}/K_m) for the four partially purified SULT isoforms, the highest value was found for SULT1A1*1 (Table 2). Thus, 3-OH-BaP was a selective, but not specific substrate for SULT1A1. Other investigators showed that the SULT1B1 protein content in liver cytosol was about one fourth that of SULT1A1 (Honma et al. 2002). The present study showed that expressed SULT1B1 had a 40-fold higher K_m value (2.0 μM) than found in human liver cytosol (0.05 μM), so it is not likely to contribute much to human liver cytosolic sulfonation of 3-OH-BaP at 0.1 μM substrate concentration (Table 2). Although SULT1A3 had activity with 3-OH-BaP, it is expressed at very low levels in the adult liver (Richard et al. 2001), and is unlikely to contribute much to 3-OH-BaP sulfonation in human liver. Since K_m values for 3-OH-BaP in human liver cytosol were similar to those of purified SULT1A1 and SULT1E1, and others have shown that SULT1A1 is expressed in liver at approximately 14-fold higher concentrations than SULT1E1 (Honma et al. 2002), we conclude that the observed activity with 3-OH-BaP in human liver cytosol is catalyzed largely by SULT1A1. Differing structural features for inhibition of SULT1A1 and SULT1E1 by OH-PCBs, discussed below, further support our conclusion that in human liver cytosol, activity with 3-OH-BaP is due primarily to SULT1A1. By chance, the three human liver cytosol fractions we used in these studies were from individuals with different SULT1A1 genotypes. One was SULT1A1*1 homozygous, a second was heterozygous for SULT1A1*1/*2 and the third was SULT1A1*2 homozygous. Kinetic analysis showed little difference between the three cytosol fractions for V_{max1}, which was 121 pmol/min/mg for the

homozygous SULT1A1*1 liver and 94 pmol/min/mg protein for the SULT1A1*2 liver (Table 1), however the small size of our sample precludes a more detailed analysis of genotype effects on 3-OH-BaP sulfonation activities.

In previous studies, we showed that OH-PCBs inhibited 3-OH-BaP sulfonation in catfish intestinal cytosol (van den Hurk et al. 2002), and that a compound structurally related to OH-PCBs, 2,4,4′-trichloro-2′-hydroxydiphenyl ether (triclosan), inhibited sulfonation and glucuronidation of 3-OH-BaP and other substrates in human liver cytosol and with SULT1A1, SULT1B1 and SULT1E1 (Wang et al. 2004). Here we demonstrated that a set of 4-OH-PCBs inhibited SULT activity with 3-OH-BaP, the major metabolite of another pollutant chemical, BaP, in human liver cytosol as well as with cDNA-expressed SULTs. In human liver cytosol, all the 4-OH-PCBs examined inhibited the sulfonation of 3-OH-BaP. Under incubation conditions in which the 3-OH-BaP substrate did not cause substrate inhibition (0.1μM 3-OH-BaP), compounds with one chlorine atom adjacent to the OH group (**B1**-**B5**) were more potent inhibitors of sulfonation than compounds in group **C**, with chlorine atoms flanking the OH group on each side. We observed very similar results for potency of inhibition and order of inhibitory potency with all three liver cytosol fractions and the two allelic variants of expressed SULT1A1. When incubated with 1.0 μM 3-OH-BaP, a concentration that produced substrate inhibition in liver cytosol and with both SULT1A1 variants, the OH-PCBs were considerably less potent inhibitors in cytosol and even more so with the expressed SULT1A1*1 and SULT1A1*2 enzymes (Table 3 and data not shown). The effect of substrate concentration on the inhibitory potency of the OH-PCBs suggested the possibility that the OH-PCBs competed with the 3-OH-BaP for an inhibitory site of the SULT1A1 protein. Gamage et al. (2003) reported that SULT1A1*2 could accommodate two molecules of the xenobiotic model substrate *p*-nitrophenol

in the active site. They proposed that substrate inhibition at high concentrations of *p*-nitrophenol was due to impeded catalysis when both binding sites were occupied. The active site of SULT1A1 appears to be plastic enough to accept a wide range of hydrophobic phenolic compounds (Gamage et al. 2003), and may be able to accommodate two molecules of 3-OH-BaP, leading to substrate inhibition, or one molecule of 3-OH-BaP and one molecule of OH-PCB, resulting in the OH-PCB inhibiting 3-OH-BaP sulfonation. The kinetic studies with 4′-OH-CB112 (compound **B5**), showed that the mechanism of inhibition was non-competitive. This result could fit the scenario for inhibition discussed above, but does not suggest direct competition of the OH-PCB for binding to the active site in an orientation that favored sulfonation. Whatever the mechanism of inhibition, the loss in inhibitory potency of OH-PCB when assays were conducted with 1.0 μM 3-OH-BaP suggested that the enzyme favored binding of 3-OH-BaP over binding of OH-PCB, and this was especially true for **C** group OH-PCBs, which showed a greater loss in potency than the **B** group compounds. These finding suggest that OH-PCBs are likely to be poor substrates for sulfonation, but this has not been studied in human liver.

We could not discern any other clear relationship of inhibitory potency with structural features or with physical-chemical properties of the OH-PCBs in this relatively small series of compounds, with cytosol or the two expressed SULT1A1 enzymes. The small size of the series of compounds studied, and lack of ready availability of a systematic series of 4-OH-PCBs prevents further analysis of structure-potency relationships at this time.

Of the other expressed enzymes studied, only SULT1E1 exhibited potent inhibition by the 4-hydroxylated PCBs. The structure-inhibitory potency requirement for SULT1E1 was very different from human liver cytosol, SULT1A1*1 or 1A1*2, where **B**-type compounds were more

potent inhibitors than **C**-type OH-PCBs. With SULT1E1, OH-PCBs with no or one *ortho* substituted chlorine were more potent as inhibitors of 3-OH-BaP sulfonation than those with two *ortho* substituted chlorine atoms. Substituted biphenyls with less than one *ortho* substituent preferentially adopt co-planar conformation of the two phenyl rings, while those with two or more *ortho* substituent atoms take non-coplanar conformations. We found a significant linear correlation between inhibitory potency and calculated solution dihedral angles (Figure 8, Table 3). Similarly, Kester et al. (2000) found that the best OH-PCB inhibitors of estrogen sulfonation (IC_{50}s < 5 nM) did not have chlorine substituents at the 2- or the 6- position. Shevtsov et al. (2003) later showed that 4,4′-di-OH-CB80 (4,4'-di-OH-3,3′,5,5′-tetrachlorobiphenyl) did not bind the SULT1E1 in a planar conformation, but rather with a 30° twist between the phenyl rings. We found that the four OH-PCBs with solution dihedral angles of 38° were more potent inhibitors than those with larger dihedral angles. While it is possible that interaction with the protein could alter the conformation of the OH-PCBs, resulting in a different dihedral angle for the enzyme-bound OH-PCB, our results show that lack of *ortho* substituents is associated with higher inhibitory potency for a xenobiotic SULT1E1 substrate, 3-OH-BaP.

SULT1A3 metabolized 3-OH-BaP with a very high V_{max}, though its preferred substrates are reported to be catecholamines and other monocyclic phenols containing hydrogen bond donors (Dajani et al. 1998). Interestingly, 50 μM OH-PCBs caused little or no inhibition of this enzyme, thereby showing that the inhibitory interaction was enzyme selective. SULT1B1, the thyroid hormone sulfotransferase, catalyzed the sulfonation of 3-OH-BaP, however OH-PCBs that were potent inhibitors of SULT1A1 were only weak inhibitors of the SULT1B1-catalyzed reaction. In contrast to results with the other enzymes, compound **A1** (6′-OH-CB35) was a fairly potent inhibitor of SULT1B1 (Table 3). Previously, *ortho*-, *meta*- and *para*-hydroxylated PCBs

were found to inhibit thyroid hormone sulfonation (Schuur et al. 1998). The *meta*-hydroxylated PCB, 3-OH-2,3′,4,4′,5-pentachlorobiphenyl (3-OH-CB118), was the most potent inhibitor of thyroid hormone sulfonation in male rat liver cytosol, followed by two *para*-hydroxylated PCBs. The *ortho*-hydroxylated PCB had the lowest potency among the four OH-PCBs studied. However, with 3-OH-BaP as substrate, the *ortho*-OH-PCB, 6′-OH-CB35, was a more potent inhibitor than those with *para*-OH groups, which suggested that the inhibitory interaction with SULT1B1 was substrate dependent.

Since several OH-PCBs have been detected in human blood, and are presumably also present in liver and other tissues, it is important to understand their biological activities. Some OH-PCBs interact with components of thyroid hormone and estrogen hormone systems (Klasson-Wehler et al. 1993; Sinjari and Darnerud 1998; Kester et al. 2000; Schuur et al. 1998). Our finding that OH-PCBs inhibited the sulfonation of 3-OH-BaP in human liver suggests another aspect of the toxicology of OH-PCBs. The interaction with phenol sulfotransferase may be of toxicological importance, as sulfonation is a major pathway of xenobiotic biotransformation (Glatt 2002). Sulfonation is particularly important at low concentrations of hydroxylated xenobiotics, such as may be encountered from environmental exposure to pollutants that require CYP-dependent biotransformation to introduce a hydroxyl group, prior to their elimination. Formation of sulfate conjugates of phenolic xenobiotics usually decreases their toxicity, so inhibition of this pathway may lead to prolonged exposure to the parent compound, a shift to an alternative phase II conjugation pathway, glucuronidation, or to further CYP-dependent metabolism. Both 3-OH-BaP and BaP-3-glucuronide bind to hemoglobin (Sugihara and James, 2003), a potentially toxic interaction. Further CYP-dependent biotransformation of 3-OH-BaP may lead to more toxic metabolites such as 3-OH-BaP-7,8-

dihydrodiol-9,10-oxide (Ribeiro et al. 1986; Glatt et al. 1987). On the other hand, xenobiotics that are activated by sulfonation, such as 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (Ozawa et al. 1998), may be rendered less toxic in the presence of inhibitors of sulfonation.

Our findings may be placed in the context of the structures of OH-PCBs that have been reported in human blood. All OH-PCB metabolites identified in blood have the hydroxy group in a *para*- or *meta*- position, with chlorine atoms on vicinal carbon atoms (Sandau et al. 2000; Sjodin et al. 2000; Hovander et al. 2002; Sandau et al. 2002). The *para*- OH-PCBs found in blood are likely to fall into the **C** group of OH-PCBs examined in this study. While these were generally less potent as inhibitors of SULT1A1 than the **B** group OH-PCBs, it is possible that the concentrations of these OH-PCBs may reach inhibitory levels in tissues of highly exposed people or animals. Sjodin et al. (2000) reported total measured OH-PCB concentrations of up to 6 μM in blood lipids, while Sandau et al (2000) reported whole blood concentrations up to 30 nM. Tissue concentrations have not been reported, but may be higher than blood levels. Type **B** OH-PCBs with the 3-chloro-4-hydroxy-substitution pattern do not appear to be persistent in blood, however, of the 209 PCB congeners, there are 19 with a 3-chloro-substitution in one of the phenyl rings, which can be biotransformed to type **B** OH-PCBs. If OH-PCBs of type **B** are formed in people, their high potency as inhibitors of 3-OH-BaP sulfonation may mean that there will be increased toxicity in people who are co-exposed to PAH and PCBs.

Conclusion

We found that several OH-PCBs, especially those with a 3-chloro-4-hydroxy-substitution pattern in the phenolic ring, inhibited the sulfonation of 3-OH-BaP in cytosol and with SULT1A1 at sub-micromolar concentrations. Some OH-PCBs with no or one *ortho* chlorine were potent inhibitors

of 3-OH-BaP sulfonation with SULT1E1. SULT1B1 and SULT1A3-catalyzed sulfonation of 3-OH-BaP were less sensitive to inhibition by OH-PCBs. The inhibitory interaction of OH-PCBs with SULT1A1 and SULT1E1 may have consequences for the biotransformation and toxicity of phenolic xenobiotics.

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Table 1. Kinetic parameters for 3-OH-BaP sulfation by Human Livers and SULT1A1*2

	K_m	K_i	V_{max1}	V_{max2}	R^2
	μM	μM	pmol/min/mg protein	pmol/min/mg protein	
HL cyt. 1	0.048	0.915	121	2	0.95
HL cyt. 2	0.051	0.534	93.0	38.50	0.956
HL cyt. 3	0.048	0.460	94.4	31.0	0.958
Mean ± S.D.	0.049 ± 0.01	0.636 ± 0.244	102 ± 15.8	28.3 ± 19.3	
SULT1A1*2	0.022	0.160	4400	290	0.953

Kinetic analysis was performed using a two-substrate model as described in *Materials and Methods*. HL cyt. 1 was homozygous for SULT1A1*1, HL cyt. 2 was heterozygous as SULT1A1*1/*2 and HL cyt. 3 was homozygous for SULT1A1*2.

Table 2. Apparent kinetic constants for cDNA-expressed sulfotransferase with 3-OH-BaP as substrate

SULTs	K_m (μM)	V_{max} (nmol/min/mg protein)	V_{max}/K_m (mL.min^{-1}.mg protein^{-1})
SULT1A1*1	0.018	6.89	383
SULT1A3	2.90	333.3	115
SULT1B1	2.00	9.70	4.9
SULT1E1	0.05	8.35	167

Partially purified SULT isoforms were used for these studies. V_{max} was calculated from the mg/ml of the partially purified preparation and corrected by the percentage of protein estimated to be SULT, from SDS-PAGE. These were 44.0% for SULT1A1*1, 39.0% for SULT1A3, 80.3% for SULT1B1 and 65.0% for SULT1E1.

Table 3. In vitro inhibition of 3-OH-BaP sulfotransferase activity by the tested OH-PCBs using human liver cytosol and cDNA-expressed sulfotransferases at 0.1 and 1.0 μM substrate concentration [a]

				IC_{50} μM							
				0.1 μM 3-OH-BaP				1.0 μM 3-OH-BaP			
	Compound	Log D at pH 7.0	Dihedral angle [°]	HL Cyt.	SULT1A1*1	SULT1A1*2	SULT1E1	HL Cyt.	SULT1A1*1	SULT1B1	SULT1E1
A1	6′-OH-CB 35	4.7	50	>100	>100	>100	~100	>100	>100	4.72	>100
B1	4′-OH-CB 35	4.7	38	0.33 ± 0.02	0.77	0.55	0.24	0.96 ± 0.30	25.2	16.8	1.02
B2	4′-OH-CB 36	4.8	38	0.67 ± 0.12	1.31	0.94	0.45	1.05 ± 0.39	28.0	37.0	1.89
B3	4′-OH-CB 69	5.1	72	0.91 ± 0.09	1.16	1.31	4.87	1.50 ± 0.32	67.5	>100	30.8
B4	4′-OH-CB 106	5.2	60	0.37 ± 0.04	1.07	1.06	1.18	2.61 ± 0.67	10.3	17.4	6.97
B5	4′-OH-CB 112	5.2	78	1.08 ± 0.12	1.17	1.48	5.35	4.22 ± 1.03	42.5	86.5	23.2
C1	4′-OH-CB 79	4.5	38	6.71 ± 0.91	6.65	4.57	0.50	58.7 ± 13.9	59.8	39.9	1.32
C2	4-OH-CB 36	4.2	38	2.30 ± 0.45	3.09	3.05	0.41	35.9 ± 1.47	>100	47.5	1.65
C3	4′-OH-CB 121	4.7	72	3.95 ± 0.23	8.15	6.52	7.98	44.6 ± 6.42	99.5	>100	16.7
C4	4′-OH-CB 159	4.7	78	1.31 ± 0.14	2.16	1.67	1.30	38.4 ± 15.2	34.1	>100	3.55
C5	4′-OH-CB 165	4.6	78	2.87 ± 0.09	2.58	2.59	6.96	47.3 ± 10.2	54.8	>100	21.4
C6	4′-OH-CB 72	4.5	57	1.72 ± 0.21	2.21	2.03	0.57	3.05 ± 0.41	33.8	>100	2.28

[a] Values for human liver cytosol are the means ± S.D. of three different livers, done in duplicate. Results for expressed SULT enzymes are the mean of duplicate determinations.

Table 4. Apparent kinetic constants for 3-OH-BaP sulfotransferase activity in human hepatic cytosol in the presence and absence of 4′-OH-CB112 (**B5**).

[**B5**]	K_m	V_{max}
μM	μM	pmol/min/mg protein
0	0.045 ± 0.02 (A)	69.1 ± 8.0 (B)
0.25	0.043 ± 0.02 (A)	56.3 ± 6.0 (B)
0.5	0.045 ± 0.01 (A)	41.2 ± 3.3 (C)
1.0	0.066 ± 0.02 (A)	33.3 ± 3.2 (D)

Values for liver cytosol are mean ± S.D. (n=3), except for studies with 0.25 μM **B5**, where two livers were used. Values followed by the same letter in parentheses are not different ($p>0.05$). Values followed by different letters are different from each other ($p<0.05$).

Legends to Figures

Figure 1. Structures of the hydroxylated PCBs used in this study: Type **A**: hydroxy without flanking chlorine atom; Type **B**: *para*-hydroxy with one flanking chlorine atom; Type **C**: *para*-hydroxy with two flanking chlorine atoms.

Figure 2. Detection of SULT1A1*1/*2 alleles by restriction fragment length polymorphism analysis. Specific PCR products were generated and digested with *Hae*II as described in Materials and Methods. Lane 1, SULT1A1*2/*2 homozygous; Lane 2, SULT1A1*1/*2 heterozygous; Lane 3, SULT1A1*1/*1 homozygous.

Figure 3. Results for rates of sulfonation of 3-OH-BaP (0.1 μM) in the presence of varying concentration of PAPS (0.125-10 μM) in human liver cytosol (A) and cytosol of SULT1A1*2 (B). Data in A were given as the mean of three experiments ± S.D..

Figure 4. Partial substrate inhibition by 3-OH-BaP in three individual human livers (A) and SULT1A1*2 (B).

Figure 5. Inhibition of 3-OH-BaP sulfotransferase in human liver cytosol by some selected OH-PCBs. Data shown in part A was with 0.1 μM 3-OH-BaP concentration and in part B with 1.0 μM 3-OH-BaP concentration. The 3-OH-BaP sulfotransferase activity was given as percentage of control. Data were the mean of three experiments ± S.D.. The structures of the OH-PCBs tested were as shown in Figure 1.

Figure 6. Inhibition of 3-OH-BaP sulfotransferase in SULT1A1*1 by some selected OH-PCBs. Part A shows results with 0.1 μM 3-OH-BaP concentration and part B with 1.0 μM 3-OH-BaP concentration. The 3-OH-BaP sulfotransferase activity was given as percentage of control. Data

were the mean of three experiments ± S.D.. The structures of the OH-PCBs tested were as shown in Figure 1.

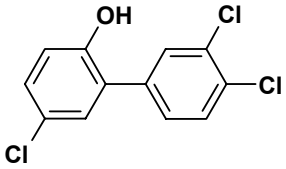
Figure 7. Inhibition of 3-OH-BaP sulfotransferase activity with SULT1A3 by OH-PCBs as shown in Figure 1. The concentration of each OH-PCB was 50 μM.

Figure 8. Correlation of IC_{50} values (μM) for each of the 4-OH-PCBs studied (groups B and C) with dihedral angles in the presence of SULT1E1. The regression line was significantly different from zero ($p< 0.001$), and the goodness of fit (r^2) was 0.73 for the positive correlation of SULT1E1 IC_{50}s with dihedral angle.

Figure 9. Effect of 4′-OH-CB112 on the kinetics of sulfotransferase with 3-OH-BaP in human liver cytosol. Graph 9a shows saturation curves, with each point representing the mean of data from three livers. The kinetic parameters are summarized in Table 4. Graph 9B shows plots of apparent K_m/V_{max} versus the concentration of 3-OH-BaP for calculation of K_i value. Data are given as the mean of three experiments ± S.D..

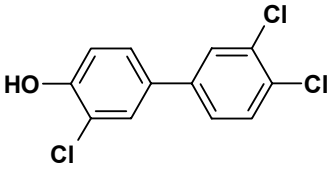
Figure 1

Type A. OH without flanking Cl

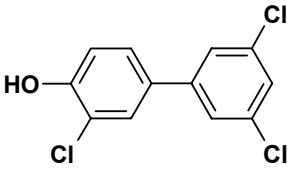


A1: 6'-OH-CB35

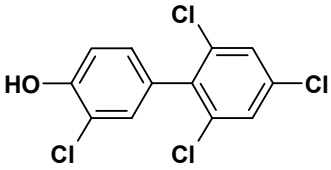
Type B. *para*-OH with one flanking Cl



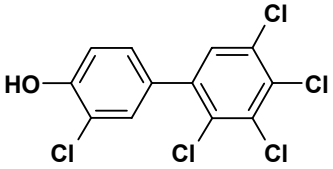
B1: 4'-OH-CB35



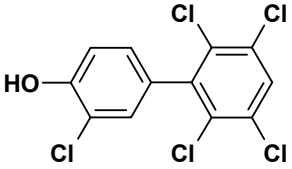
B2: 4'-OH-CB36



B3: 4'-OH-CB69

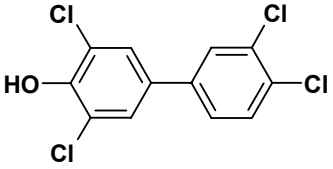


B4: 4'-OH-CB106

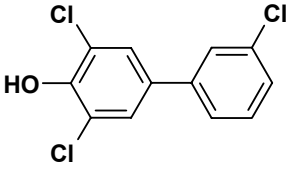


B5: 4'-OH-CB112

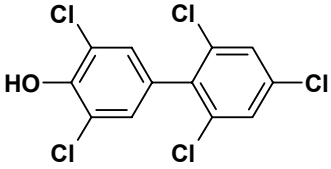
Type C. *para*-OH with two flanking Cls



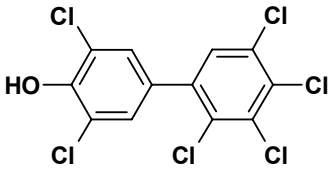
C1: 4'-OH-CB79



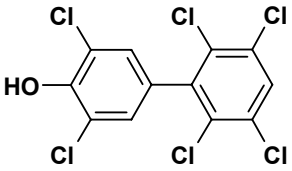
C2: 4-OH-CB36



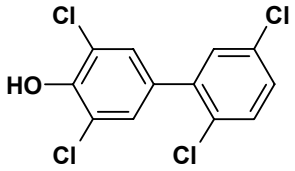
C3: 4'-OH-CB121



C4: 4'-OH-CB159



C5: 4'-OH-CB165



C6: 4'-OH-CB72

Figure 2

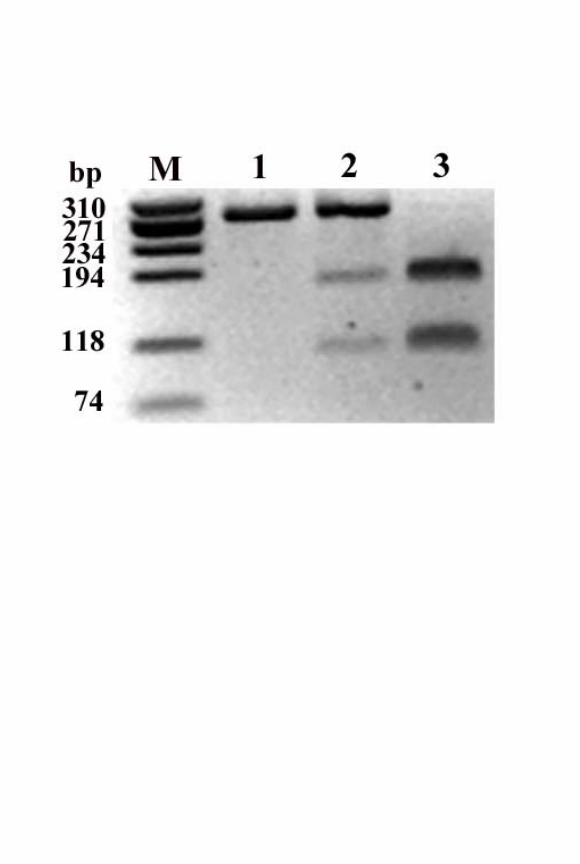


Figure 3

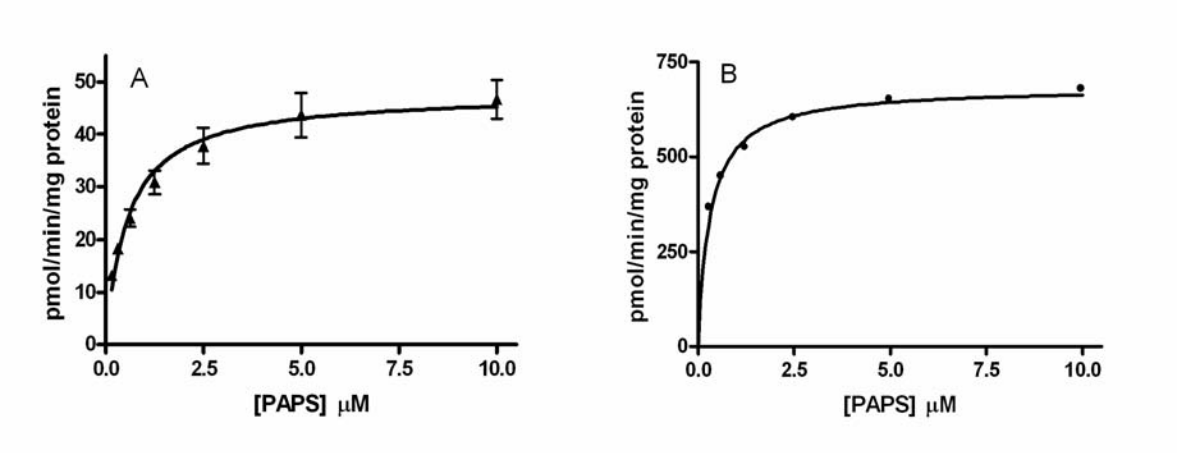

A
pmol/min/mg protein
50
40
30
20
10
0
0.0
2.5
5.0
7.5
10.0
[PAPS] μM
B
pmol/min/mg protein
750
500
250
0
0.0
2.5
5.0
7.5
10.0
[PAPS] μM

Figure 4

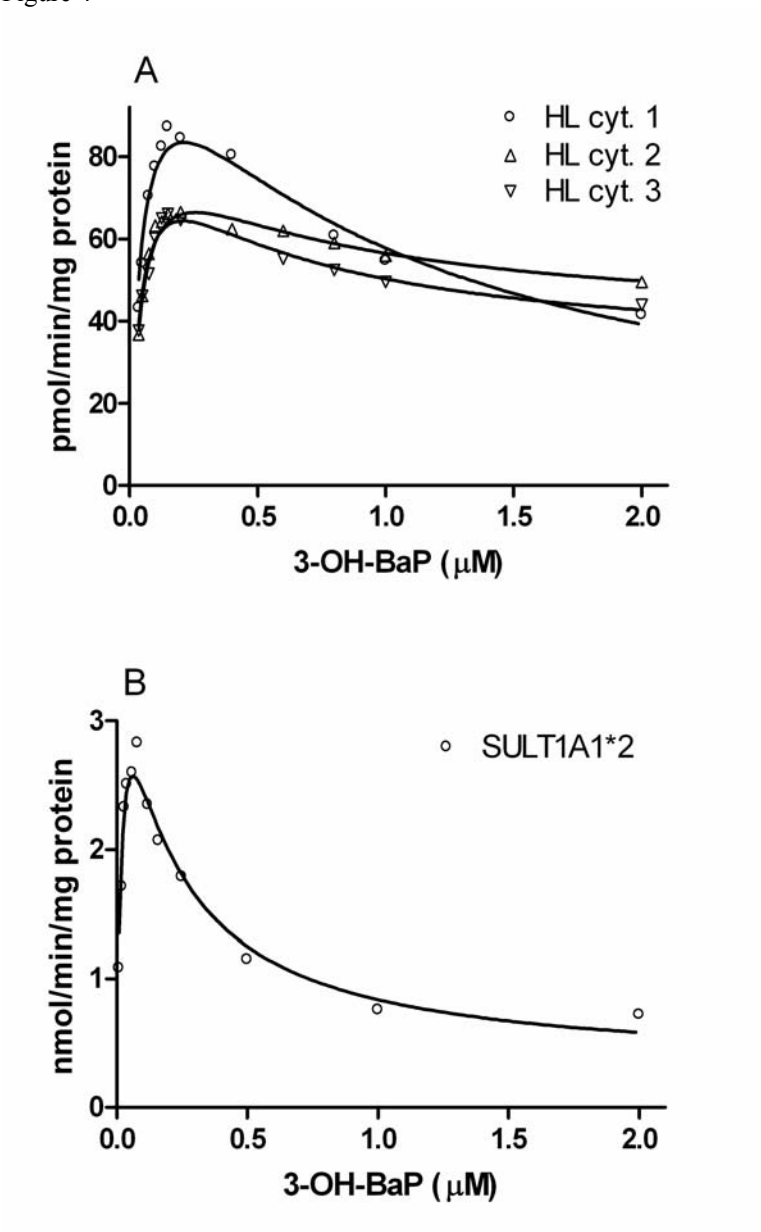

A
HL cyt. 1
HL cyt. 2
HL cyt. 3
pmol/min/mg protein
0
20
40
60
80
0.0
0.5
1.0
1.5
2.0
3-OH-BaP (μM)
B
SULT1A1*2
nmol/min/mg protein
0
1
2
3
0.0
0.5
1.0
1.5
2.0
3-OH-BaP (μM)

Figure 5

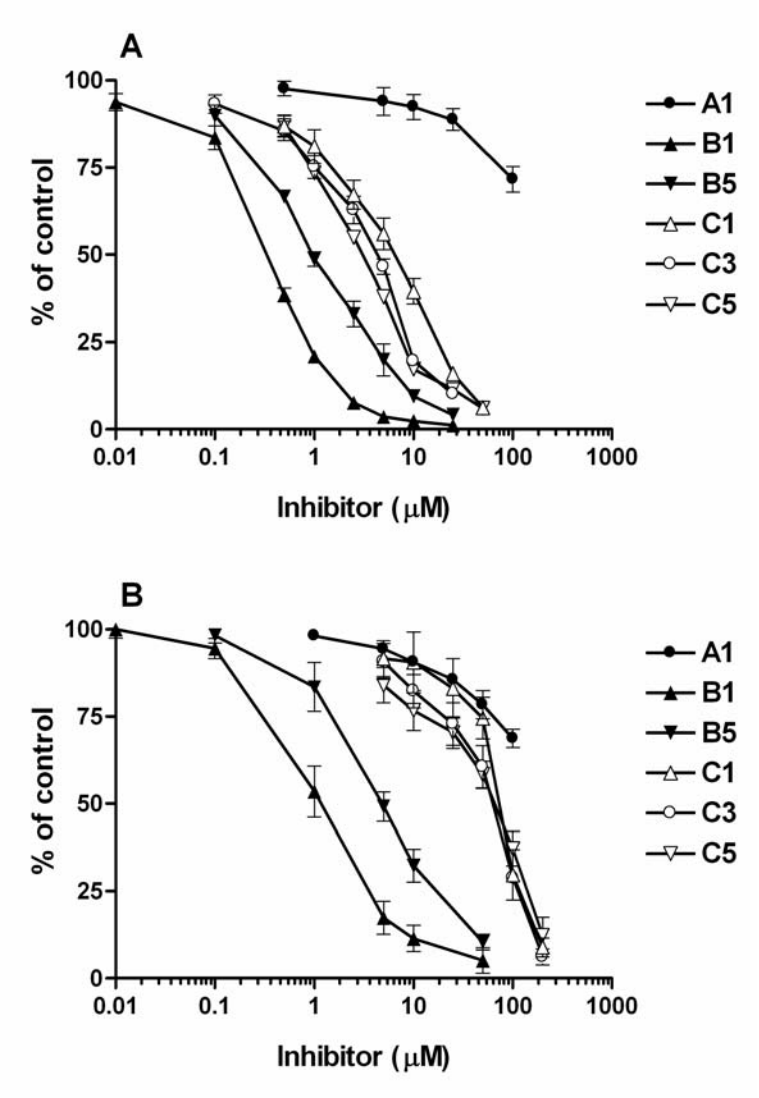

A
100
75
50
25
0
% of control
0.01
0.1
1
10
100
1000
Inhibitor (μM)
A1
B1
B5
C1
C3
C5
B
100
75
50
25
0
% of control
0.01
0.1
1
10
100
1000
Inhibitor (μM)
A1
B1
B5
C1
C3
C5

Figure 6

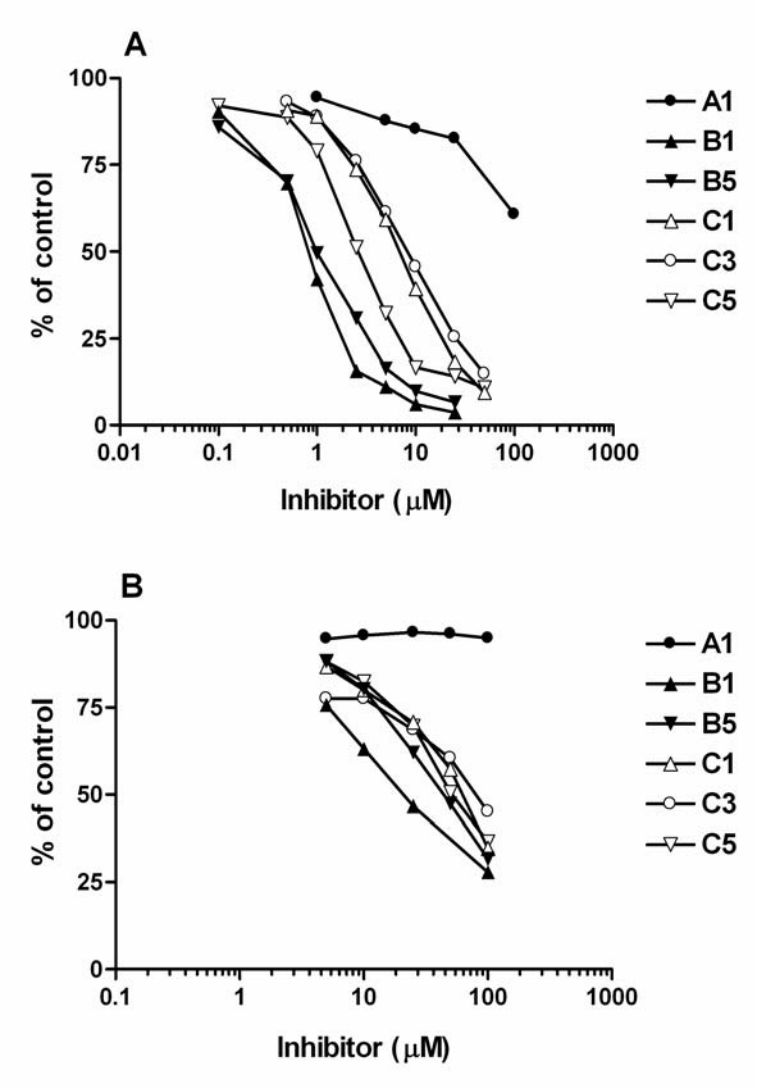

A
100
75
50
25
0
% of control
0.01
0.1
1
10
100
1000
Inhibitor (μM)
A1
B1
B5
C1
C3
C5
B
100
75
50
25
0
% of control
0.1
1
10
100
1000
Inhibitor (μM)
A1
B1
B5
C1
C3
C5

Figure 7

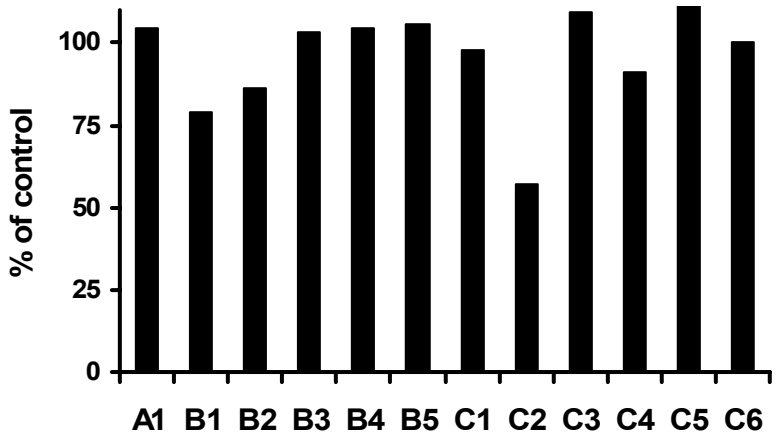


Figure 8

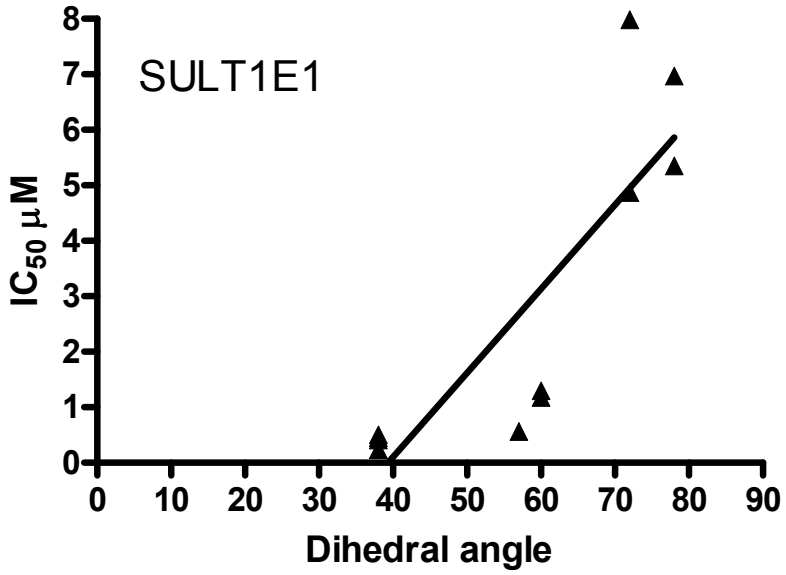
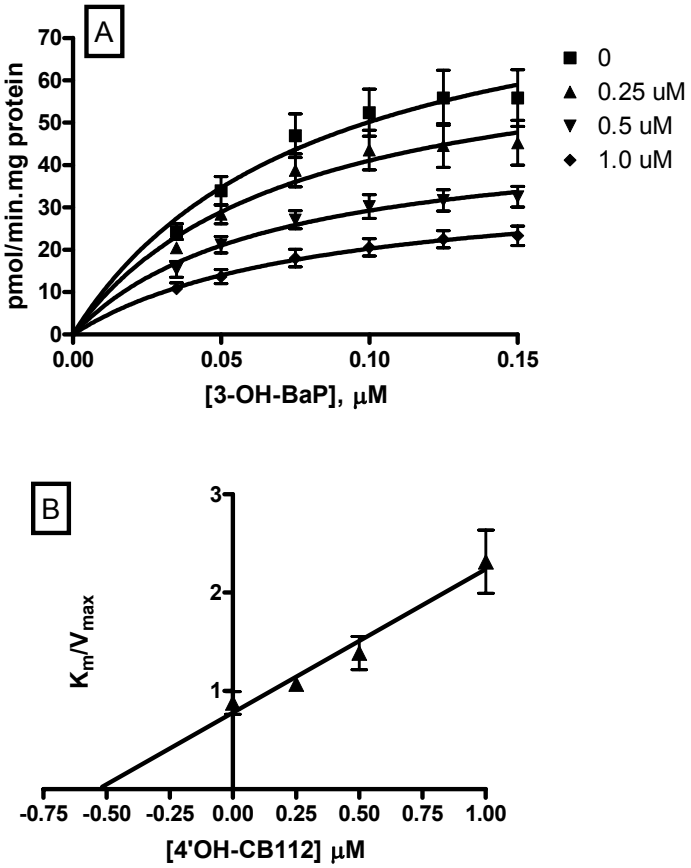

SULT1E1
IC50 µM
Dihedral angle
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
70
80
90

Figure 9


A
pmol/min.mg protein
70
60
50
40
30
20
10
0
0.00
0.05
0.10
0.15
[3-OH-BaP], μM
0
0.25 uM
0.5 uM
1.0 uM
B
K_m/V_{max}
3
2
1
-0.75
-0.50
-0.25
0.00
0.25
0.50
0.75
1.00
[4'OH-CB112] μM